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Nutritional Comparison of Transgenic and Local Cowpea Grown in Nigeria

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ABSTRACT: Cowpea, a common nutritious legume in Nigeria, faces significant productivity challenges from the pod-boring pest, *Maruca vitrata.* This study investigated the nutritional differences between genetically modified varieties expressing *Cry1Ab* (a recombinant insecticidal toxin) and local cowpea varieties. Four cowpea varieties, including a transgenic variety (Sampea-20T), its non-transgenic counterpart (Sampea-10), and two improved varieties (Iron Beans and Ife Brown), commonly found in Nigerian markets, were evaluated for proximate, mineral and amino acid profiles using standard techniques. Sampea-20T and Sampea-10 were substantially similar in terms of crude protein, crude fiber, and crude fat, with slight differences in ash and carbohydrate content. Sampea-20T significantly differed from Ife brown and iron beans cowpea varieties in multiple nutritional components. Ife brown had a significant mineral profile, while Sampea-20T and Sampea-10 were similar except for potassium (K) and magnesium (Mg). Sampea-20T had notably higher levels of histidine, isoleucine, and leucine compared to the other varieties but lower levels of tryptophan. Sampea-20T had considerably higher levels of five out of nine non-essential amino acids compared to Sampea-10, along with comparable quantities to Ife brown and iron beans in four amino acids. This study has shown that there were no biologically significant differences ($p<0.05$) found among the compositional parameters measured in cowpea samples taken from the transgenic cowpea (Sampea-20T), its non-transgenic counterpart (Sampea-10), and local commercial varieties (Ife brown and Iron beans).

Keywords: Transgenic cowpea, Sampea 20-T, Sampea-10, *Bacillus thuringiensis* (Bt)

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INTRODUCTION

Cowpea (*Vigna unguiculata* ssp. *unguiculata*) (L. Walp.), a member of the *Fabaceae* family, is an edible leguminous plant primarily grown in Africa, with Nigeria being the largest producer (Ogu and Owoeye, 2013; Ibrahim et al., 2017; Aletan, 2019). For communities with limited access to essential nutrients from animal sources, cowpea serves as a significant source of high-quality protein, minerals, and vitamins (Aletan, 2019; Gondwe et al., 2019). Cowpea is commonly cultivated in the tropics and subtropics of Africa, Latin America, Southeast Asia, and the United States. It is a drought-tolerant crop that can thrive in a variety of climates and soil types (Appiah et al., 2011; Gondwe et al., 2019). In Africa, cowpea is predominantly cultivated in West and Central Africa, with Nigeria being the world's largest producer (Bolarinwa et al., 2021). Nigeria boasts the largest collection of cowpea germplasm, with more than 15,000 landraces and over 2,000 wild relatives (Fatokun et al., 2018; Then et al., 2022). Cowpea exhibits morphological and nutritional diversity, as seen in various regional variants (OECD, 2015; Madar et al., 2017; OECD, 2018). Despite its resistance to most biotic and abiotic stresses, the bean pod borer (*Maruca vitrata*) poses a significant threat, leading to yield losses of 20–80% (Addae et al., 2020; Ba et al., 2019). To control *M. vitrata*, farmers frequently resort to insecticide sprays five to eight times per season, despite the negative economic, health, and environmental consequences, as no cowpea cultivars resistant to it are currently known (Addae et al., 2020). In 2019, Nigeria approved the cultivation and commercialization of Pod-borer resistant (PBR) cowpea, a transgenic variety expressing Cry1Ab, an insecticidal recombinant toxin. Cry1Ab, also known as Bt toxin, is derived from the soil bacteria *Bacillus thuringiensis* (Bt) and is designed to protect plants from *M. vitrata* larvae, a pod-borer that feeds on cowpea (BCH, 2019). When comparing transgenic crops to their conventional counterparts, compositional evaluation aims to identify significant changes in nutritional composition. The compositional evaluation of transgenic crops seeks to identify any substantial differences in nutritional composition compared to their conventional equivalents and assess the safety of intentional or unintended modifications (Swamy et al., 2019). The safety assessment approach, based on the concept of substantial equivalence, evaluates whether foods and feeds derived from transgenic crops are as safe as their conventional counterparts (Oh et al., 2021; Caradus, 2023). Natural variations in nutritional components of conventional crops, which have a history of safe use, must be considered when determining whether there are significant differences in the nutritional components of GM crops compared to their conventional counterparts (CAC/GL 45-2003).

While natural variations in nutritional components among plant cultivars may occur randomly and pose no harm to human health, consistently lower or higher concentrations may raise safety concerns despite their natural variation (Kuiper et al., 2003; Oh et al., 2021). In this study, partial compositional analyses were conducted on samples of transgenic cowpea (Sampea-20T), its non-transgenic counterpart, Sampea-10, and two morphologically distinct commercial varieties, iron beans and brown beans (Ife Brown), sold in Nigeria. The aim was to determine whether the genetic modification leading to a cowpea variety expressing Cry1Ab resulted in any biologically significant changes in proximate, mineral content, and amino acid profiles.

MATERIALS AND METHODS

Study area

The study area was Abuja, the Federal Capital Territory (FCT) of Nigeria, with geographical coordinates of 9° 3′ 28.26″ N and 7° 29′ 42.29″ E.

Sample collection

The four cowpea varieties were used, including a transgenic variety (Sampea 20-T), its non-transgenic counterpart, Sampea-10, and two improved varieties (Iron Beans and Ife Brown) (Figure 1) were purchased from major markets in Abuja, FCT, Nigeria. All the reagents utilized in this study were of analytical grade. The experimental analysis was conducted at Rofem Analytical Laboratory, Tanke, Ilorin, Kwara State, Nigeria.

Proximate analysis:

After being dried at 105°C until a consistent weight was reached, the moisture content of the different cowpea types was calculated (Swamy et al., 2019). The moisture content (%) was calculated using equation (1). Based on the techniques described in AOAC (2000), the ash content was calculated (gravimetrically). Equation (2) was used to calculate the percentage of ash in the samples. The total nitrogen and crude protein (N×5.95) were calculated using the micro-Kjeldahl method (AOAC, 2000). Crude fats were extracted with petroleum ether using a Soxhlet system, and total carbohydrate was determined using the difference method (summing the values of moisture, ash, crude protein, and crude fat and deducting the sum from 100) (AOAC, 2000).

$$Moisture\ (\%) = \frac{Weight\ of\ original\ sample - Weight\ of\ dried\ sample}{weight\ of\ original\ sample} \times 100 \quad (1)$$

$$Ash\ Content\ (\%) = \frac{Weight\ of\ sample + crucible - Weight\ of\ ash + crucible}{weight\ of\ sample} \times 100 \quad (2)$$

Mineral analysis

Flame Atomic Absorption Spectrometry was used to analyze minerals (AOAC, 2005). Each sample's ash was digested with nitric acid and boiled to produce a clear digest. Each solution was filtered and diluted to a maximum of 50 ml. The concentration of each mineral was extrapolated in triplicates using the associated calibration curves for each mineral and a blank sample.

Amino-acid profile

The method reported by AOAC (2005) was modified to determine the amino acid profile in the cowpea samples. Samples were dried to a consistent weight, defatted in a chloroform/methanol solution, hydrolyzed in boiling sulfuric acid, evaporated in a rotary evaporator, and placed into an Applied Biosystems PTH Amino Acid Analyser. Since tryptophan breaks down during acid

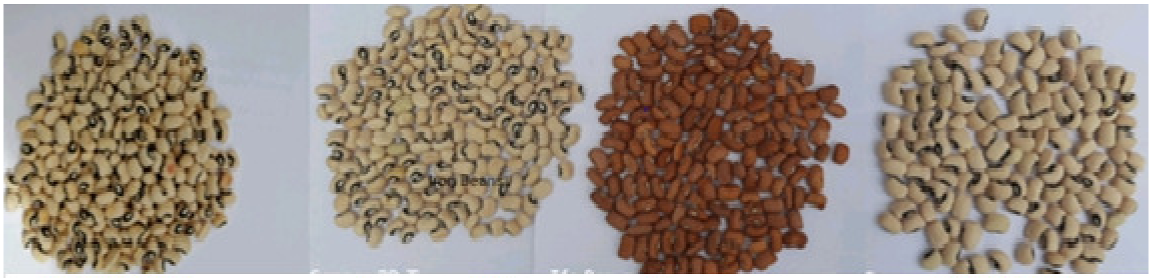

Figure 1: Four varieties of cowpea analyzed in this study

Table 1: Proximate composition of four varieties (transgenic and local) of cowpea.

Proximate Content (%)	Sampea-10	Sampea-20T	Iron Beans	Ife Brown	Literature Range
Moisture	6.65 ± 0.20^{b}	6.46 ± 0.01^{a}	7.05 ± 0.08^{c}	6.86 ± 0.01^{b}	5.20 - 14.20
Total Ash	2.23 ± 0.05^{b}	2.47 ± 0.03^{a}	2.99 ± 0.04^{b}	2.76 ± 0.01^{b}	3.20 - 4.10
Crude protein	24.74 ± 0.29^{b}	24.99 ± 0.06^{b}	22.98 ± 0.04^{a}	26.21 ± 0.04^{c}	16.00 - 31.00
Crude fibre	3.13 ± 0.02^{c}	3.10 ± 0.03^{c}	2.89 ± 0.02^{b}	2.65 ± 0.02^{a}	1.33 - 10.70
Crude fat	1.78 ± 0.04^{ab}	1.68 ± 0.03^{a}	1.87 ± 0.01^{b}	1.78 ± 0.03^{ab}	0.50 - 4.48
Carbohydrate	59.73 ± 0.54^{a}	61.31 ± 0.04^{b}	62.23 ± 0.06^{c}	59.75 ± 0.01^{a}	30.00 - 74.80

Values are expressed as mean ±SEM (n = 2); Values with different superscripts across rows are significantly different from each other at p<0.05.

hydrolysis, samples containing it were hydrolyzed using 4.2M NaOH following the procedure outlined by Yust et al. (2004).

Statistical analysis

All determinations underwent analysis in duplicate, and the results are presented as mean ± SEM. The one-way Analysis of Variance (ANOVA) was performed using the GraphPad Prism Statistical software program. The significance of the differences was defined as p<0.05. The Turkey Post-test was used to compare the mean difference.

RESULTS AND DISCUSSION

Proximate composition

The results for the proximate (moisture, ash, crude proteins, fiber, and carbohydrate) analysis of Sampea 20-T, Sampea-10, Ife Brown, and Iron Beans are presented in (Table 1). While the moisture content of Sampea 20-T at 6.46% is significantly lower than that of Ife Brown (7.05%) and Iron Beans (6.86%), it is similar to the 6.67% observed in its non-transgenic counterpart, Sampea-10, as reported by Magashi et al (2019). This suggests that differences are more likely due to variety variations than a direct consequence of genetic modification. Maintaining moisture levels below 10% in most food grains almost halts respiration, reducing the risk of bacterial and fungal infestations and increasing grain storage life (Sujeetha, 2014; Aziz et al., 2022; Kumar et al., 2023). The moisture content of Sampea 20-T is suitable for long-term storage. Ash content in biological material indicates the amount of essential minerals present (Ismail, 2017). In this study, the ash content of Sampea 20-T at 2.47% was lower than that of Ife Brown and Iron Beans at 2.76% and 2.99%, respectively, but similar to Sampea-10 (2.2%), indicating substantial equivalence to its non-transgenic counterpart. Crude protein levels were similar for Sampea 20-T and Sampea-10 and were observed to be higher than in Iron Beans but lower compared to Ife Brown. Similar to the moisture and ash parameters, the crude protein content in Sampea 20-T at 24.99% was similar to the 24.10% determined for Sampea 10, as reported by Magashi et al (2019. In terms of crude fiber, Sampea 20-T had significantly higher levels at 3.1% compared to Iron beans (2.89%) and Ife Brown (2.65%), but was similar to Sampea-10 at 3.13%. Crude fats were similar for all varieties and were within the ranges reported in the literature (Owolabi et al., 2012; Ailenokhuoria and Omolekan, 2019). The proximate analysis showed that Sampea-20T was substantially equivalent to Sampea-10 in terms of crude protein, crude fiber, and crude fat, with slight deviations in other proximate parameters measured. Furthermore, all proximate parameters analyzed for Sampea 20-T were found to be within the

Table 2: Mineral composition of four varieties (transgenic and local) of cowpea.

Mineral Content	Cowpea Varieties				Literature range
	Sampea-10	Sampea-20T	Iron Beans	Ife Brown	
Ca (%)	0.41 ± 0.002[b]	0.40 ± 0.0015[b]	0.32 ± 0.0040[a]	0.42 ± 0.0010[c]	0.03 – 1.01
K (%)	0.03 ± 0.004[b]	0.023 ± 0.0010[a]	0.03 ± 0.0000[b]	0.04 ± 0.0005[c]	0.957-2.15
Na (%)	0.13 ± 0.001[a]	0.13 ± 0.0015[a]	0.12 ± 0.0020[a]	0.03 ± 0.0010[b]	-
Mg (%)	0.14 ± 0.004[c]	0.12 ± 0.0030[b]	0.13 ± 0.0020[b]	0.12 ± 0.0010[a]	0.0905 - 0.374
Zn (mg/100g)	7.40 ± 0.010[a]	7.30 ± 0.0040[a]	6.65 ± 0.0210[a]	7.48 ± 0.0020[ab]	0.23 – 6.9
Fe (mg/100g)	1.02 ± 0.0050[a]	0.99 ± 0.0090[a]	1.01 ±0.0010[b]	1.05 ± 0.0035[c]	0.48 – 135.6
Cu (mg/100g)	2.20 ± 0.002[b]	2.23 ± 0.0015[b]	1.98 ± 0.0145[a]	2.40 ± 0.0030[c]	0.5 – 2.2
Mn (mg/100g)	0.97 ± 0.0025a	1.02 ± 0.0415[a]	0.27 ±0.004[b]	0.72 ± 0.0020[c]	1.7 – 4.3
Pd (mg/100g)	-	0.014 ± 0.0020[a]	0.060 ±0.003[b]	0.0155 ± 0.0055[a]	-

Values are expressed as mean ±SEM (n = 2); Values with different superscripts across rows are significantly different from each other at p<0.05.

Table 3: Amino acid profiles of four varieties (transgenic and local) of cowpea.

Essential amino acid (g/100g)	Sampea-10	Sampea-20T	Ife Brown	Iron Beans	Literature range
Histidine	2.30 ± 0.02[b]	3.50 ± 0.05[d]	2.50 ± 0.04[a]	3.20 ± 0.02[c]	2.0 - 4.5
Isoleucine	4.80 ± 0.22[a]	5.50 ± 0.04[c]	4.40 ± 0.04[a]	5.10 ± 0.04[b]	2.8 - 5.4
Leucine	8.34 ± 0.01[b]	8.57 ± 0.05[d]	7.73 ± 0.03[a]	9.56 ± 0.05[c]	5.7 - 11.3
Lysine	5.10 ± 0.02[a]	5.40 ± 0.06[ac]	5.20 ± 0.04[a]	5.80 ± 0.03[b]	3.9 - 8.1
Methionine	1.38 ± 0.04[b]	1.19 ± 0.02[c]	0.85 ± 0.00[a]	1.07 ± 0.05[c]	0.9 - 3.5
Phenylalanine	5.50 ± 0.04[b]	5.60 ± 0.05[bc]	4.90 ± 0.01[a]	4.90 ± 0.05[a]	4.4 - 10.6
Threonine	5.07 ± 0.05[b]	4.94 ± 0.03[db]	4.07 ± 0.05[a]	5.48 ± 0.04[c]	3.2 - 5.9
Tryptophan	1.09 ± 0.01[b]	0.95 ± 0.03[c]	1.28 ± 0.02[a]	1.20 ± 0.015[a]	0.7 - 1.5
Valine	5.30 ± 0.04[b]	5.60 ± 0.06[bc]	6.10 ± 0.06[a]	6.00 ± 0.05[a]	3.4 - 6.3
Non-essential amino-acids (g/100g)	**Sampea-10**	**Sampea-20T**	**Ife Brown**	**Iron Beans**	**Literature range**
Alanine	5.20 ± 0.02[b]	4.60 ± 0.04[c]	3.80 ± 0.02[a]	4.80 ± 0.06[c]	3.4 - 5.1
Arginine	5.90 ± 0.09[b]	6.70 ± 0.00[d]	5.20 ± 0.04[a]	6.10 ± 0.05[cb]	6.4 - 10.8
Aspartic acid	8.50 ± 0.08[b]	9.69 ± 0.14[cd]	6.89 ± 0.04[a]	9.68 ± 0.10[c]	6 – 13
Cystine	1.20 ± 0.0[a]	1.20 ± 0.03[a]	1.30 ± 0.03[a]	1.20 ± 0.03[a]	-
Glutamic acid	10.8 ± 0.05[b]	12.3 ± 0.08[a]	11.70 ± 0.18[a]	12.22 ± 0.11[a]	14.1 - 19
Glycine	4.45 ± 0.03[b]	4.16 ± 0.05[d]	3.72 ± 0.02[a]	4.77 ± 0.05[c]	3.1 - 4.8
Proline	3.40 ± 0.06[a]	3.40 ± 0.05[a]	3.50 ± 0.05[a]	3.70 ± 0.0[a]	4 - 8.9
Serine	4.72 ± 0.02[b]	5.12 ± 0.04[a]	5.15 ± 0.05[a]	4.84 ± 0.03[b]	3 - 5.8
Tyrosine	3.20 ± 0.09[b]	3.90 ± 0.09[cd]	2.30 ± 0.09[a]	4.00 ± 0.09[c]	2.6 - 4.5

Values are expressed as mean ±SEM (n = 2); Values with different superscripts across rows are significantly different from each other at p<0.05.

range of cowpea varieties reported in the literature (Akande and Fabiyi, 2010; Alphonsus and Felix, 2012; Owolabi et al., 2012).

Mineral composition of cowpea

The results of the mineral composition of all cowpea varieties are presented in (Table 2). Among the macro-minerals analyzed, Ca was the most abundant in all varieties, with levels ranging from 0.316% to 0.42%, while K was the least abundant (0.028% to 0.037%) in all cowpea varieties. Owolabi et al. (2012) observed similar findings when they compared the proximate and mineral composition of five cowpea varieties in Nigeria. Ife Brown had significantly higher levels of Ca and K but the least amounts of Na (0.033%) when compared to Sampea-20T (0.13%), Sampea-10 (0.13%), and Iron beans (0.124%). Regarding micro-minerals, Fe was the most abundant with levels ranging from 9.97 mg/Kg to 10.53 mg/Kg, while Mn was the least abundant with levels from 0.27 mg/Kg to 1.02 mg/Kg. Additionally, Ife Brown had higher levels of Zn, Fe, and Copper but lower levels of Mn compared to Sampea-20T and Sampea-10. This study showed that Sampea-20T had similar mineral levels to Sampea-10. Substantial equivalence was observed, with high mineral levels that slightly varied with Ife Brown and Iron beans but within the levels reported in the literature (Akande and Fabiyi, 2010; Alphonsus and Feli, 2012; Boukar et al., 2015).

Amino-acid profile of cowpea

Table 3 provides the amino acid profiles of the four cowpea varieties analyzed. Leucine was the most abundant essential amino acid in all varieties, while tryptophan was the least abundant essential amino acid for all varieties, except in the Iron Beans variety, where methionine was the least abundant essential amino acid. Sampea-20T had slightly higher levels of three essential amino acids (histidine, isoleucine, leucine) and lower levels of methionine and tryptophan compared to Sampea-10. Both had similar levels of lysine, phenylalanine, threonine, and valine. Sampea-20T compared favorably to Ife Brown and Iron Beans with histidine, isoleucine, methionine, and phenylalanine levels but had lower levels of tryptophan and valine compared to both. For the non-essential amino acids, glutamic acid was the most abundant, while cystine was the least abundant in all varieties. Sampea-20T had significantly higher levels in five of the nine non-essential amino acids, namely, arginine, aspartic acid, glutamic acid, serine, and tyrosine, when compared to Sampea-10. Both had similar levels of cystine and proline. Sampea 20-T had similar levels of alanine, cystine, glutamic acid, and proline when compared to Ife Brown and Iron Beans, while the three varieties differed in levels of arginine and glycine. Overall, the amino acid profiles of Sampea 20-T were within the range of variation reported in the literature (Owolabi et al., 2012; OECD, 2018).

Conclusion

In this study, no biologically significant differences were found among the compositional parameters, including proximate, minerals, and amino acids profiles measured in cowpea samples taken from the transgenic cowpea Sampea-20T and its non-transgenic counterpart Sampea-10, as well as local commercial varieties Ife Brown and Iron Beans. The compositional parameters measured in samples of Sampea-20T cowpea were within or similar to ranges reported in the literature for conventional cowpea varieties, including Ife Brown and Iron Beans, with a history of safe consumption. Overall, no recurrent patterns indicated that genetic modification had resulted in biologically significant modifications to the composition or nutritional value of Sampea-20T grains. In this study, and based on the parameters analyzed, Sampea-20T was found to be compositionally equivalent to Sampea-10, its non-transgenic variety, and within a comparable composition of commercial cowpea, including Ife Brown and Iron Beans, all of which have a long history of safe use. These findings further expanded the evidence supporting the safety and nutritional equality of genetically modified cowpeas, particularly the Sampea-20T variety. Further research and regulatory assessments can continue to build confidence in the use of such genetically modified crops in food production and agriculture.

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